# Remodelling of actin cytoskeleton in mammalian sperm during capacitation *in vitro*

# Změna uspořádání aktinového cytoskeletu ve spermii savců v průběhu kapacitace *in vitro*

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Bakalářská práce
Praha 2008

Školitelka: RNDr. Hortová Kateřina, PhD.

Děkuji své školitelce RNDr. Kateřině Hortové, PhD. za ochotu a trpělivost, se kterou mi pomáhala s vypracováním mé práce.

## *Content*

## *Abstract*

In order the fertilization of an egg could occur, spermatozoa have to reside in female reproductive tract and undergo series of biochemical modifications called capacitation. This provides sperm with ability of the acrosome reaction and penetration of the egg. Sperm capacitation is accompanied with a massive actin polymerization in anterior part of the sperm head and the acrosome reaction induces fast F-actin breakdown allowing the sperm outer acrosome membrane and plasma membrane to fuse. It has been suggested that protein tyrosine phosphorylation represents an important regulatory pathway during the sperm capacitation and there is an evidence for enhancing of actin polymerization by this mechanism. Thus, the actin cytoskeleton plays an important role in egg fertilization. It is responsible for plasma membrane modification and it represents the last barrier before the acrosome reaction can occur.

## Keywords

Capacitation, actin, microfilaments, mammalian sperm, tyrosine phosphorylation

## *Abstrakt*

Aby byly savčí spermie schopny oplodnit vajíčko musí setrvat nejprve druhově specifickou dobu v samičím reprodukčním traktu a podstoupit řadu biochemických změn označovaných jako kapacitace. Pouze kapacitované spermie mohou prodělat akrozomální reakci a vnikout do vajíčka. Kapacitace je doprovázena aktinovou polymerizací v přední části hlavičky spermie zatímco před nástupem akrozomální reakce dochází k rychlému zhroucení této aktinové sítě umožňující spojení vnější akrozomální a plazmatické membrány. Předpokládá se, že tyrozinová fosforylace proteinů je jedna z důležitých regulačních cest během kapacitace, a že je zodpovědná za polymerizaci aktinu ve spermii. Aktinový cytoskelet hraje tedy důležitou roli během přípravy spermie k oplodnění. Je zodpovědný za změny probíhající v oblasti plazmatické membrány spermie a je také poslední překážkou před nástupem akrozomální reakce.

## Klíčová slova

Kapacitace, actin, microfilamenta, savčí spermie, tyrozinová forforylace

## *Introduction*

To reach fertilization two gametes have to fuse and form a zygote. The female gamete is one of the largest cells in the organism and is called the egg (or ovum). The male one is named the sperm (or spermatozoon, pl. spermatozoa), it is highly motile and often the smallest cell in the organism. Gametes spring up during meiosis, when four haploid cells arise from one diploid progenitor cell. The division together with proliferation, differentiation of the cell and formation of special structures is called spermatogenesis for sperm and oogenesis for eggs. Spermatogenesis occurs in seminiferous tubules of the testis and continues in epididymis where sperm are stored. Sperm undergo more biochemical and morphological changes later on both in male and female reproductive tract.

To become capable of fertilization sperm have to capacitate what enables them to undergo the acrosome reaction following binding to zona pellucida, the egg's coat. Hydrolytic enzymes are released and sperm penetration can occur resulting in fusion of gametes. Molecular mechanisms of these processes are not well known yet but it is obvious that the cell cytoskeleton plays an important role in them. In this work I have concentrated on the role of actin cytoskeleton in capacitation.

## *The Cytoskeleton*

The cytoskeleton is a highly dynamic and adaptable system of filaments in eucaryotic cells. It is used for pulling the chromosomes apart at mitosis, splitting the dividing cell as well as for the intracellular transport. It protects cell from mechanical stress, allows cell to move and controls the cell shape. There are three main types of filaments: intermediate filaments, microtubules and microfilaments.

Intermediate filaments

Intermediate filaments provide mechanical strength of cell. They form a protective cage for DNA in the nucleus called the nuclear lamina and strong rope-like structures 10 nm in diameter in the cytosol. They are made up from elongated and fibrous subunits forming lateral contacts between α-helical coiled coils. Due to this fact intermediate filaments tolerate stretching and bending.

Microtubules

Microtubules support the intracellular transport and determine the positions of membrane-enclosed organelles. They form cilia and flagella on the cell surface and during cell division a bipolar mitotic spindle. Microtubules consist of 13 protofilaments forming a hollow cylindrical structure with a 25 nm diameter. Protofilaments are formed from protein subunits of tubulin, a heterodimer consisting of two globular proteins called $\alpha$-tubulin and $\beta$-tubulin. Tubulin assembles head-to-tail creating polar filaments with minus end ($\alpha$-subunit end) and plus end ($\beta$-subunit end). Minus ends are anchored in a microtubule-organizing center whereas plus ends are growing towards the periphery.

Microfilaments

Microfilaments (or actin filaments) are invoved in cell motility and determine the shape of the cell. They mediate cell shape changes during mitosis, play role in contractile activities, separation of daughter cells, cell-cell and cell-substrate interactions, endocytosis, organelle positioning, secretion and transmembrane signalling. They form lamellipodia and filopodia used for exploring territory and for moving around. The subunits are globular polypeptides assembling head-to-tail to form polar filaments which form one helical structure. Actin filaments are the thinnest with 7 nm in diameter which provide them with flexibility as well as fragility. The highest concentration of actin filaments is in the cell cortex, a layer under the plasma membrane where filament nucleation most frequently occurs.

## Actin

Actin (43kDa) exists either in a monomeric (G-actin) or in a polymeric (F-actin) form. Each actin molecule can bind ATP, which is hydrolyzed to ADP during polymerization. Polymers assemble spontaneously via noncovalent interactions. Actin filaments are structurally polarized with a possibility to grow on both ends. The plus end grows more quickly than does the minus end. Nucleation sites for actin filaments contribute to the structure of focal adhesions and adherent junctions.

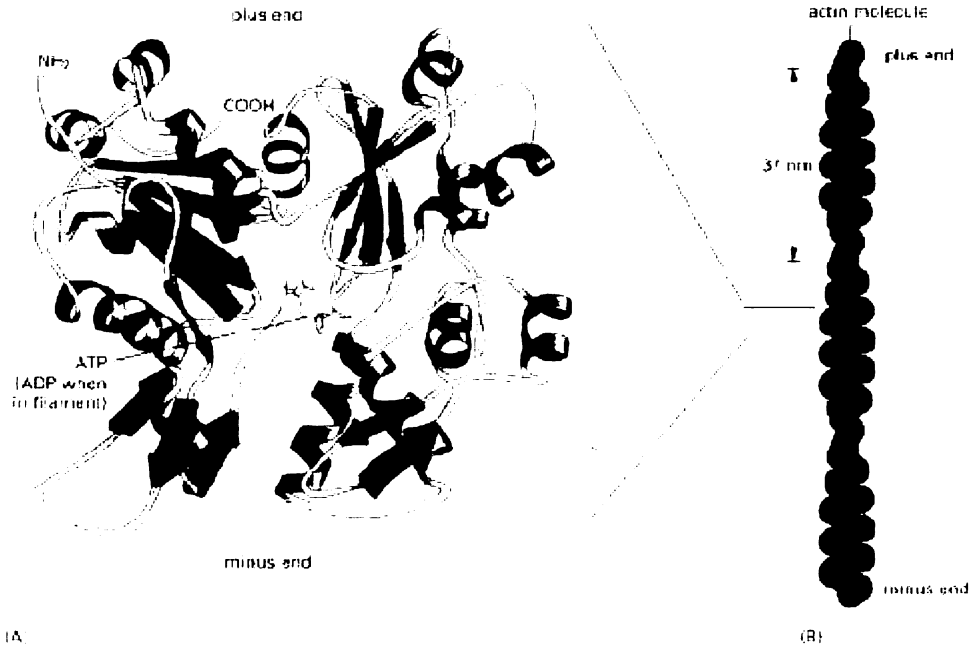


Figure 1: An actin monomer and actin filament. (A) An actin monomer with ATP/ADP binding site and plus and minus ends. (B) Polarized actin filament (adopted from Molecular Biology of the Cell, Alberts et al.).

A large number of actin-binding proteins regulate actin assembly by controlling filament formation and cross-linking of the actin network. The activities of these proteins are often modulated by signalling molecules such as $Ca^{2+}$ or phosphorylated phosphoinositides. Proteins regulating filament formation include actin monomer binding proteins (e.g. profilin), actin filament capping proteins (e.g. capping protein, CP) and $Ca^{2+}$ dependent severing proteins (e.g. gelsolin, villin, fragmin, adseverin, and scinderin). Cofilin (actin depolymerising factor, ADF) is a less efficient severing protein regulated by phoshorylation.

Superorganization of actin polymers is mediated by actin side-binding or cross-linking proteins (e.g. α-actinin, filamin, fimbrin, and villin). The movement of actin filaments within the cell is controlled by myosins.

Rho-like GTPases are involved in signalling pathways that link extra-cellular growth signals or intracellular stimuli to the assembly and organization of the actin cytoskeleton (summarized Schmidt and Hall, 1998).

## *Sperm*

The spermatozoon has two parts: the head with haploid DNA, the strong tail (or flagellum) to reach the egg, joined together by the neck. Both the tail and the head are surrounded by the plasma membrane forming structurally and functionally distinct membrane domains without using intercellular junctions. The plasma membrane is found to consist of at least three distinct domains: the acrosomal region, the post-acrosomal region and the plasma membrane of the flagellum. Many alterations occur during the transit down the epididymis and up the female reproductive tract. Since transmembrane proteins are stabilized by linkage through membrane skeleton proteins to cytoskeletal structures, such associations may be important for defining the boundaries and contents of different sperm surface domains. Components of the membrane skeleton complex that are possibly involved in producing domains include actin, myosin and spectrin (summarized Eddy and O´Brien, 1994; Millette, 1999).

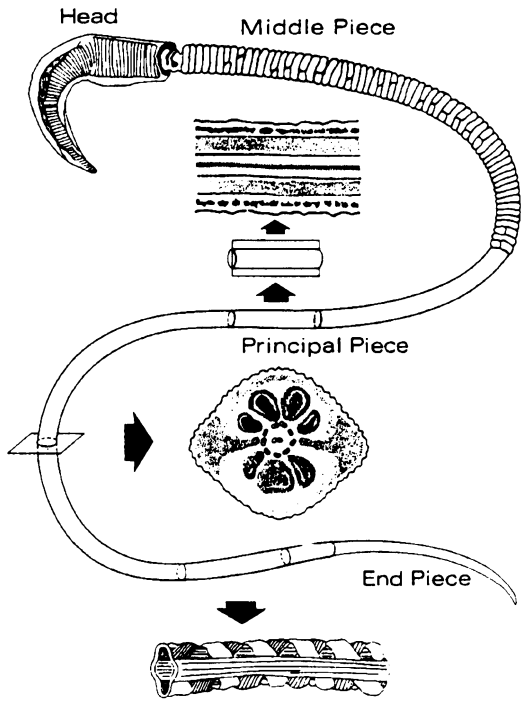


Figure 2: Features of the mammalian spermatozoon (adopted from Eddy and O´Brien, 1994).

The length of mammalian spermatozoa vary between species. Human spermatozoa have a total length of approximately 60-70 μm, whereas mouse and rat sperm have a length of about 130 and 190 μm, respectively. It is also of note that in contrast to most somatic cells, in

which 80-90 % of the cell is water, mammalian sperm are about 50 % dry matter (Millette, 1999 and citation therein).

## The Head

The main part of the head is the nucleus. It includes an extremely tightly packed haploid DNA associated with protamines instead of histones. Sperm transcription is shut down. Pores in the nuclear envelope are absent except for the most posterior part of the nucleus. Inner surface of the nucleus is lining the nuclear lamina, which allows anchoring of the chromatin. On the top of the head overlying the anterior end of the nucleus is the acrosomal vesicle originate as a vesicular extension of the Golgi apparatus. It contains hydrolytic enzymes to help penetrating the egg's outer coat and others to help fusing the plasma membrane of the egg and to assist in cortical granules exocytosis. The release of the acrosomal vesicle is called the acrosome reaction and occurs after a sperm-egg contact has taken place. The cytoskelet lies between the acrosome and the nucleus and just under the plasma membrane. The shape of the head in mammals varies from falciform (rodents) to ovate (human, guinea pig and rabbit) (summarized Eddy and O´Brien, 1994; Millette, 1999).

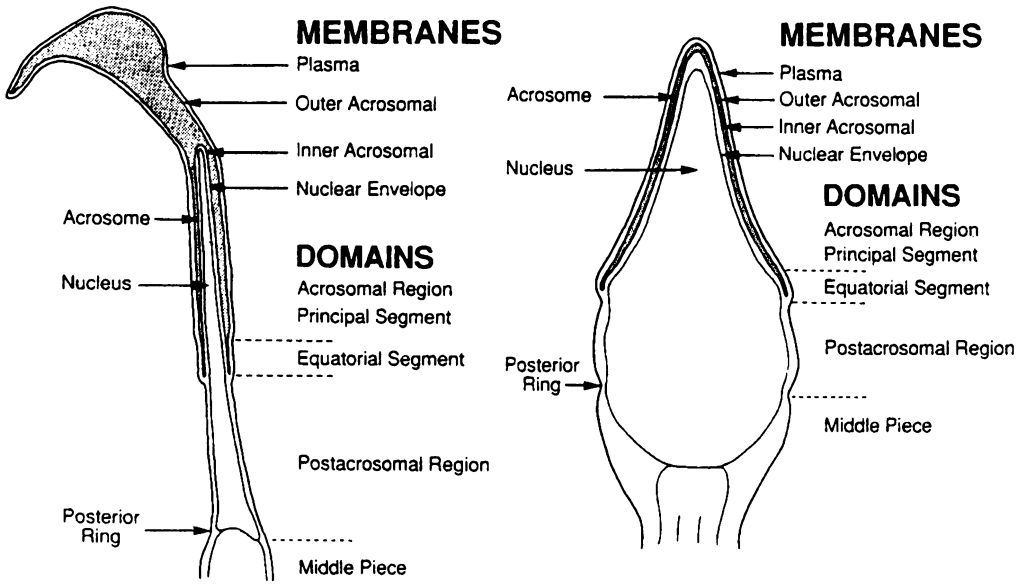


Figure 3: The head of guinea pig and human spermatozoon (adopted from Eddy and O´Brien, 1994).

## The Tail

To fulfil their function of fertilization sperm are equipped with a flagellum a motile part of sperm. The tail is subdivided into four sections: the connecting piece, the middle piece, the principal piece, and the end piece (see figure 2). The movement of sperm is handled by the core of the tail called axoneme. The mitochondrial sheath is wrapped tightly around the outer dense fibers in the middle piece and it is replaced by the fibrous sheath in the principal piece of the tail. After the fibrous sheath ends the tail constricts quickly. The mammals' sperm flagellum is further surrounded by nine outer dense fibers lying between the axoneme and the mitochondrial later fibrous sheath. Outer dense fibers originate at the base of nucleus and proceed through the middle piece extending to about 60 % of total length of the principal piece. Human flagella are about 55 μm long. Chinese hamster sperm tail can exceed up to 250 μm (summarized Eddy and O'Brien, 1994; Millette, 1999).

## The cytoskeleton

The cytoskeleton of the sperm head is composed of three parts: sub-acrosomal, post-acrosomal and para-acrosomal cytoskeleton (see figure 3). The sub-acrosomal cytoskeleton (or perforatorium) is situated between the inner acrosomal membrane and the outer nuclear membrane. It is a major structure in rodent spermatozoa and contains cysteine-rich proteins. It is thought to have a mechanical role in egg penetration. The post-acrosomal cytoskeleton is located between the nucleus and the plasma membrane posterior to the acrosome. It contains an ordered array of filaments. It is slightly more prominent in spatulate sperm where it might help to maintain the asymmetric shape. Sub-acrosomal and post-acrosomal cytoskeletons are referred to as the perinuclear theca, which is similar to the nuclear matrix of somatic cells. It is believed that perinuclear theca has an important role in the overall nuclear shape. An additional role of the perinuclear theca might be to link together and stabilize the association of the acrosome, nucleus, and post-acrosomal plasma membrane. The para-acrosomal cytoskeleton is located between the anterior tip and convex surface of the acrosome and the plasma membrane of falciform sperm and it is formed of filaments similar in size to intermediate filaments. In the nucleus there is the nuclear lamina which is lining the inner surface of the nuclear envelope and forms part of the nuclear skeletal network anchoring the chromatin. The lamins have extensive sequence homology with intermediate filament proteins and apparently belong to the same family of structural proteins (summarized Eddy and O'Brien, 1994; Millette, 1999).

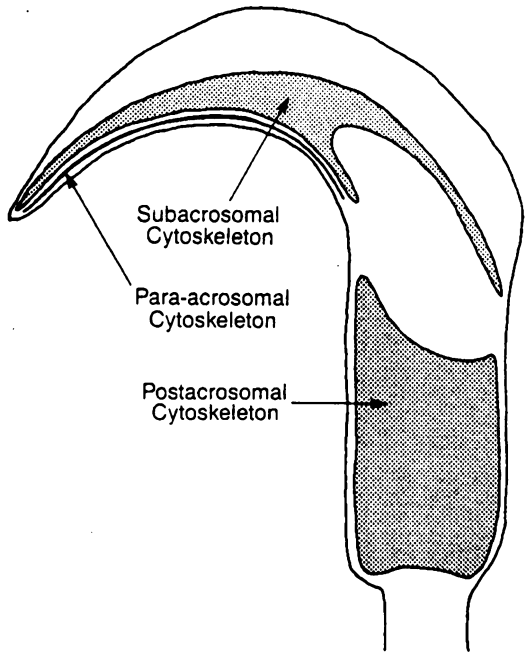


Figure 4: Cytoskeleton of head of the hamster spermatozoon (adopted from Eddy and O'Brien, 1994).

The tail cytoskeleton is consisting of the axoneme, the outer dense fibers and the fibrous sheath. Within the axoneme microtubules form a "9 + 2" array. Two central microtubules are encircled by nine doublets of microtubules. The doublet consist of one complete microtubule with 13 protofilaments and one incomplete closed by its apposition to the wall of the complete tubule. Dynein is found in the arms extending from the microtubular doublet towards the incomplete tubule of the adjacent ring doublet. The bending of the flagellum is provided by sliding of adjacent microtubule doublets driven by dynein motor proteins. They have ATPase activity and move unidirectional towards the minus end of microtubules. The ATP for dynein is produced by mitochondria from the mitochonodrial sheath.

The fibrous sheath lies immediately subjacent to the sperm plasma membrane, but is not attached to it. There are two longitudinal columns connected by circumferential ribs. The fibrous sheath defines the general shape of the sperm flagellum. As a unit, the fibrous sheath is thought to regulate the spatial plane in which the flagellum beats. Since the fibrous sheath is physically attached to some of the outer dense fibers its involvement in microtubular sliding must be minimal.

Nine outer dense fibers are reported to contain keratin-like proteins rich in cysteine that are increasingly cross-linked by disulfide bonds during epididymal maturation. It is

unlikely that the outer dense fibers play an active role in flagellar motion but they might be determining the elastic properties of the sperm tail (summarized Eddy and O´Brien, 1994; Millette, 1999).

## Sperm and actin

Actin is known to be important in the transformation of rounded spermatids into elongated spermatids with a clearly defined head and tail, and is particularly involved in shaping the acrosomal region of the sperm head (Scarlett et al., 2001). Microfilaments situated in the connecting piece/post-nuclear region of sperm might be instrumental in determining the angle of orientation between the head and the tail (Clarke et al., 1982). Actin polymerization and depolymerization plays an important role in membrane fusion during acrosome reaction (Liu et al., 2002). It could be involved in the surface modifications that accompany epididymal maturation, capacitation, and acrosome reaction of bovine spermatozoa (Howes et al., 2001; Moreno-Fierros et al., 1992) and it may be involved in generation of the flagellar beat (Clarke et al., 1982).

There are many studies about localization of actin in mammalian spermatozoa but the answer is still unclear. Actin was found in a number of mammalian species including hamster, boar, rabbit, mouse, rat, ram, guinea pig, bull, monkey, human, etc. Differences in results are given by highly dynamic actin remodelling, alterations between species as well as by different methods of detecting and staining used by researches.

Actin was detected in the acrosomal region of guinea pig (Moreno-Fierros et al., 1992), hamster (Fouquet and Kann, 1992; Talbot and Kleve, 1978), boar (Castellani-Ceresa et al., 1992), rabbit (Camatini et al., 1988), and human (Liu et al., 1999).

In rabbit, mouse, rat, monkey, and human actin was detected between the nucleus and the acrosome of spermatids. In rat, monkey, and humans epididymal sperm cells actin remained undetected (Fouquet and Kann, 1992).

Monomeric actin was described in the post-acrosomal region of human sperm (Clarke et al., 1982; Ochs and Wolf, 1985) but there is evidence for filamentous actin in rat epididymal spermatozoa in the sub-acrosomal space and in the post-acrosomal region as well (Vogl et al., 1993). F-actin was observed with mouse and human uncapacitated sperm in post-acrosomal space and in the tail of mouse sperm. No increase of F-actin in tail occur after capacitation of mouse and human sperm but there was in bull and ram sperm (Brener et al., 2003).

Monomeric actin was observed with the boar sperm plasma membrane and actin filaments with the outer acrosomal membrane (Peterson et al., 1978; Peterson et al., 1990). F-actin was observed with bovine sperm membrane domains (Spungin et al., 1995; Yagi and Paranko, 1995). It was also find in both the acrosomal cap region and the principal piece region of the sperm plasma membrane in ejaculated human spermatozoa indicating that cytoskeleton may regulate the maintenance of these surface assemblies (Virtanen et al., 1984).

Actin was detected in the tail of guinea pig spermatozoa (Moreno-Fierros et al., 1992) and in the connecting and principal piece of hamster (Talbot and Kleve, 1978) and boar sperm (Castellani-Ceresa et al., 1992). Actin was observed with the whole length of rat tail (de las Heras et al., 1997), in the neck of rabbit, in the principal piece of mouse and bovine epididymal spermatozoa (Fouquet and Kann, 1992; Howes et al., 2001), and in the connecting piece of human sperm tail (Clarke et al., 1982).

## *Capacitation*

Spermatozoa ejaculated by fertile mammals are not able to fertilize immediately, in order to penetrate an egg. Ejaculated sperm should undergo biochemical and biophysical changes called capacitation which enable sperm to undergo the acrosome reaction and to develop hyperactivated motility in appropriate conditions. Capacitation is brought about by the milieu of the female tract during their transport to the site of fertilization in the ampulla of the Fallopian tube (Bedford and Cross, 1999). Capacitation was first observed by Austin and Chang in 1951 independently. They have referred to some physiological changes of the spermatozoa in the female genital tract before they are capable of penetrating and fertilizing the eggs (Chang, 1984). The first demonstration of capacitation *in vitro* was the report of Yanagimachi and Chang in 1963 (Yanagimachi, 1994).

Sperm capacitation is temperature dependent. An incubation temperature of 37°C to 38°C is adequate to support *in vitro* capacitation in most cases. The minimum time for capacitation could be less than 1 hour in some species (e.g., mouse, cat, and perhaps human), whereas in some other species it can take several hours (e.g. rabbit). Ejaculated spermatozoa of many species are more resistant to *in vitro* capacitation than are epididymal spermatozoa. This is likely because the plasma membrane of ejaculated spermatozoa is more stable than that of epididymal spermatozoa (Yanagimachi, 1994).

*In vitro* capacitation can be reached in defined capacitation media with differences among species (e.g. heparin for bovine sperm capacitation). In most cases, capacitation media contain energy substrates (e.g. pyruvate, lactate and glucose), a cholesterol acceptor (e.g. BSA), $HCO_3^-$, $Ca^{2+}$, low concentration of $K^+$, and physiological $Na^+$ concentration (Visconti et al., 2002).

During capacitation modifications in sperm surface protein distribution and alterations in plasma membrane characteristics occur. Among these changes is cholesterol efflux leading to plasma membrane destabilization. Receptors for zona pellucida proteins appear on the sperm head. Elevation of intracellular bicarbonate and calcium occur leading to activation of adenylyl cyclase (AC) (this is described latter). Higher bicarbonate concentration could be responsible for rise of pH. Influx of $Na^+$ and efflux of $Cl^-$ and $Zn^+$ occur. A set of sperm proteins is phosphorylated on membrane tyrosine residues during capacitation. In mouse and bovine spermatozoa has been observed an increase in sperm membrane potencial from about –30 to about –60 mV. Increase in sperm metabolism occur (summarized Baldi et al., 1996; Bedford and Cross, 1999; Eddy and O´Brien, 1994).

## Capacitation and actin

During capacitation of boar, bull, ram, mouse, and human sperm time-dependent polymerization of globular G-actin to filamentous F-actin occurs mainly in the sperm head (Brener et al., 2003; Castellani-Ceresa et al., 1992; Castellani-Ceresa et al., 1993). By enhancing intracellular $Ca^{2+}$ concentrations actin is depolymerised, resulting in acrosomal exocytosis (Brener et al., 2003).

The actin polymerization during capacitation is stimulated by protein tyrosine phosphorylation (Brener et al., 2003) of a subset of mouse sperm proteins of $M_r$ 40,000-120,000 (Visconti et al., 1995a) as well as bovine proteins of the same size (Galantino-Homer et al., 1997). It is associated with presence of BSA, $Ca^{2+}$ and $HCO_3^-$ in the capacitation medium (Visconti et al., 1995a).

Both protein tyrosine phosphorylation and capacitation appear to be regulated by cAMP-dependent protein kinase A (PKA) (Lefievre et al., 2002; Visconti et al., 1995b). Adenylyl cyclase (AC) is stimulated by bicarbonate (Garty and Salomon, 1987) or $Ca^{2+}$ (Hyne and Garbers, 1979) and/or calmodulin ($Ca^{2+}$ depending protein) resulting in rise of cAMP level and stimulation of PKA (Brener et al., 2003). The plasma membrane destabilization results in changes of permeability of $Ca^{2+}$ and $HCO_3^-$. Destabilization is given by cholesterol efflux for which bovine serum albumin (BSA) serve as an extra-cellular sink (Davis et al., 1980; Davis and Gergely, 1979). Since PKA is not capable of phosphorylation on tyrosine residues, an intermediate sperm tyrosine kinase (STK) may be involved. There are three possible mechanisms: stimulation of a tyrosine kinase by PKA; inhibition of a phosphotyrosine phosphatase; and phosphorylation of proteins by PKA on serine or threonine residues which leads these proteins to subsequent phosphorylation of tyrosine residues (Visconti et al., 1995b; Visconti et al., 2002).

Epidermal growth factor (EGF) found in the female reproductive tract stimulates protein tyrosine phosphorylation (Brener et al., 2003) and actin polymerization during capacitation (Spungin et al., 1995). Reactive oxygen species stimulate protein tyrosine phosphorylation suggesting that $H_2O_2$ activates AC (Aitken et al., 1995). $H_2O_2$ can substitute for $HCO_3^-$ (Rivlin et al., 2004).

Galantino-Homer et al. (2004) described alternative hypothesis that elution of cAMP levels is maintained by cyclic nucleotic phosphodiesterase (PDE) and AC does not play an important role in bovine sperm capacitation but this theory was ruled out. Similarly like Lefievre et al. (2002) proved for human sperm capacitation and acrosome reaction Galantino-

Homer et al. (2004) showed that PDE is providing a constitutive activity therefore can not control cAMP levels.

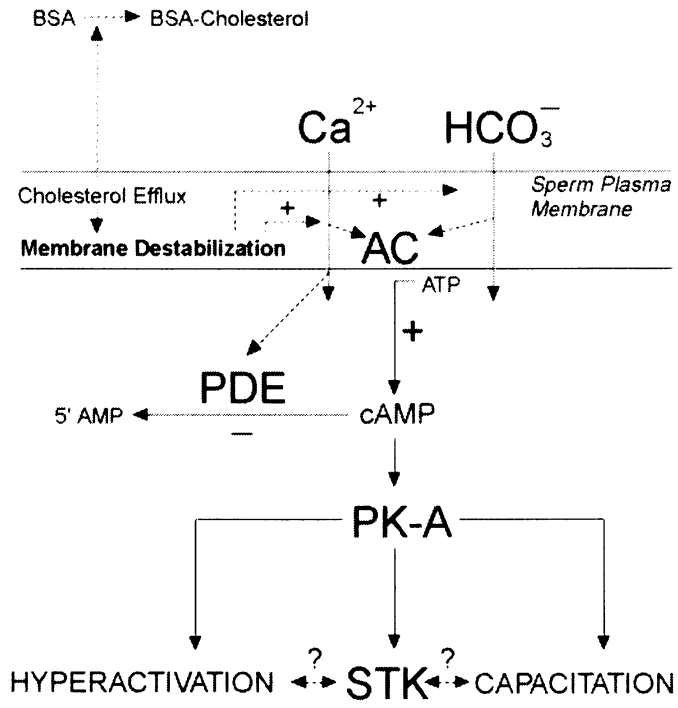


Figure 5: Described in the text (adopted from Visconti et al., 1995b).

Cohen et al. (2004) demonstrated a role of phospholipase D (PLD) in the of actin polymerization and capacitation in spermatozoa. PLD is activated by PKA or protein kinase C (PKC) pathways during capacitation or acrosome reaction respectively. The data indicated that PKC is activated when PKA activity is blocked, and activation of PKA leads to inhibition of PKC. PKC is activated towards the end of capacitation leading to F-actin breakdown and acrosome reaction (Cohen et al., 2004).

PKCα is forming a complex with PLD1 in the cytoplasm. After activation of PKCα by lysophosphatic acid (LPA) presented in the female reproductive tract PKCα is translocated to the sperm plasma membrane and PLD1 to the particule fraction suggesting outer acrosomal membrane. PLD1 is subsequently producing phosphatidic acids (PA) by hydrolysis of phospholipids. PA is a fusogenic compound that may stimulate the fusion between the outer acrosomal and the plasma membrane leading to acrosomal exocytosis (Cohen et al., 2004; Garbi et al., 2000).

Inhibition of PKC is maintained by PKA through inhibiting of phospholipase C (PLC). PLC hydrolyze the phophatidyl-inositol phosphate (PIP) and phosphatidyl-inositol 4,5-bisphosphate ($PIP_2$), to generate diacylglycerol (DAG), the PLC activator, and inositol 1,4,5-

trisphosphate ($IP_3$) which mobilises $Ca^{2+}$ from intracellular stores (Spungin et al., 1995). $PIP_2$ is a cofactor for PLD, thus, when PLC is blocked by PKA, $PIP_2$ is not hydrolyzed and PLD activation occur. Thus, it is possible that AC or PKA is partially inactivated towards the end of the capacitation which allows PLC activation leading to PKC activation and achieving the acrosome reaction (Cohen et al., 2004).

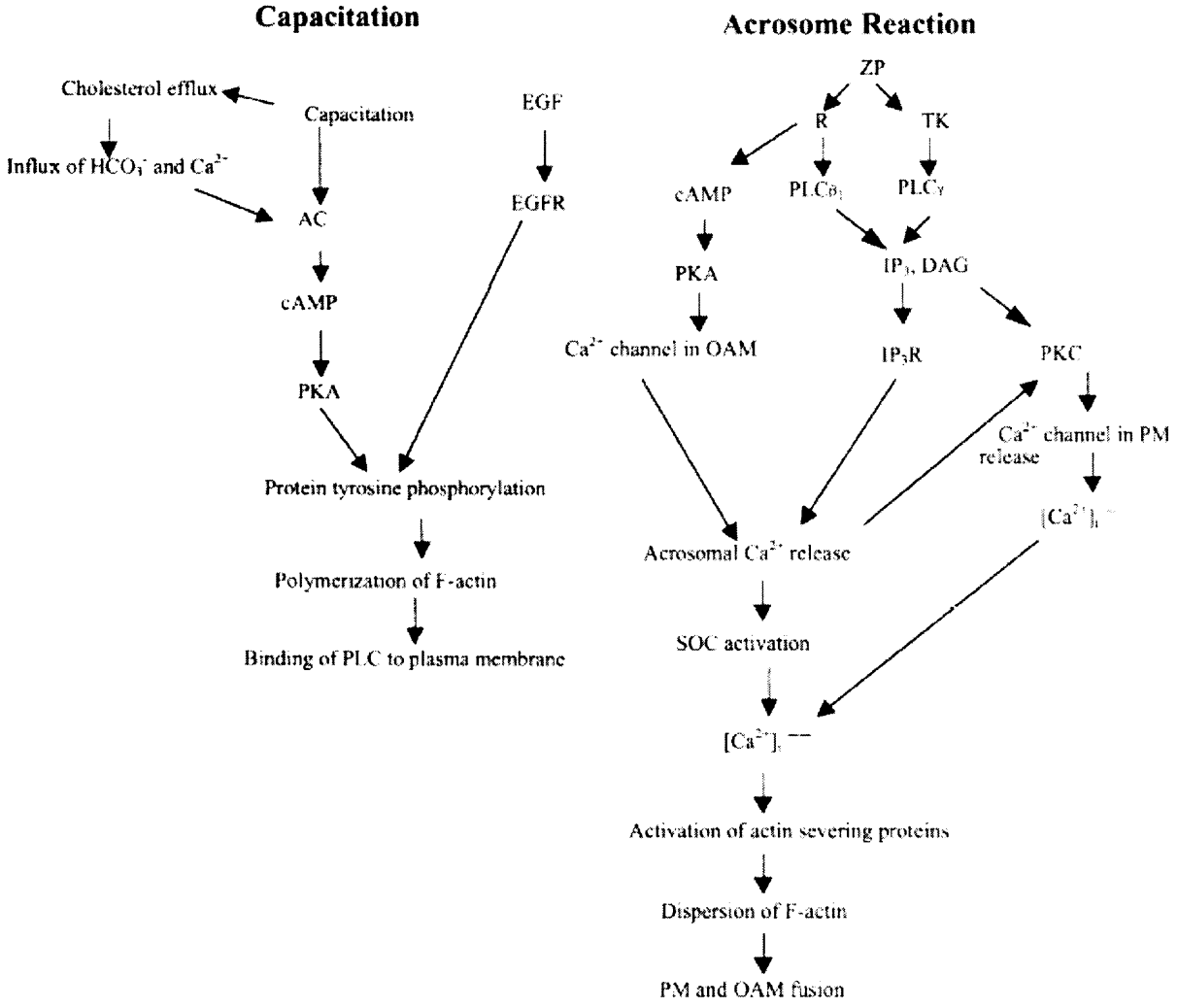


Figure 6: (R) receptor, (TK) tyrosine kinase, (OAM) outer acrosomal membrane, (SOC) store-operated calcium, (PM) plasma membrane (Breitbart, 2002)

EGF from female reproductive tract binds to EGF receptor which autophosphorylate following a rapid F-actin assembly between the plasma and outer acrosomal membranes. The activated receptors phosphorylate PLCγ allowing the enzyme to bind to membrane bound F-actin. This transfer of PLC from the cytosol to the membrane cytoskeleton is important for the acrosome reaction. After the sperm-egg contact sperm receptors enable small elevation of

intracellular calcium which activates the membrane bound PLCγ, allowing it to hydrolyze membrane lipids (PIP and $PIP_2$), which enhance the membrane fusibility. The activated PLC also hydrolyzes PIP and $PIP_2$ bound to various actin severing proteins, thus releasing them from the membrane. This is then followed by a second rise in intracellular calcium which activates the actin severing proteins, leading to dispersion of the F-actin network between the plasma and outer acrosomal membranes enable them to fuse (Spungin et al., 1995).

Recently more investigations of these problems are handled. Etkovitz et al. (2007) investigated two alternative pathways of phosphatidylinositol 4-kinase ($PI_4K$) activation. There are two ways of activation: indirect by PKA and mediated by phosphatidylinositol 3-kinase ($PI_3K$); and direct by PKC. $PI_4K$ produces $PIP_2$, a known cofactor for PLD activation, and $PI_3K$ produces $PIP_3$. High activity of PKA, downregulates PKC activity and activates $PI_3K$ while relatively high activity of PKC activates $PI_4K$ (Etkovitz et al., 2007).

Sperm binding to the zona pellucida causes further activation of cAMP/PKA and PKC, respectively. PKC opens a calcium channel in the plasma membrane. PKA together with $IP_3$ activate calcium channels in the outer acrosomal membrane, which leads to an increase in cytosolic calcium. The depletion of calcium in the acrosome will activate a store-operated calcium entry mechanism in the plasma membrane, leading to a higher increase in cytosolic calcium, resulting in F-actin dispersion which enable the outer acrosomal and the plasma membrane to come into contact and fuse completing the acrosomal reaction (Breitbart, 2003).

The balance between F- and G-actin is controlled by actin-binding proteins. A number of them such as calicin (von Bulow et al., 1995), thymosin 10 (monomer-sequestering protein), destrin (actin depolymerizing protein), TS-ACP (testis-specific actin capping protein) (Howes et al., 2001), CP β3 (β subunit of the heterodimeric actin-binding "capping protein") (von Bulow et al., 1997), myosin, spectrin, vimentin (Virtanen et al., 1984), α-actinin, tropomyosin (Yagi and Paranko, 1995), gelsolin (Cabello-Agueros et al., 2003; de las Heras et al., 1997), scinderin (Pelletier et al., 1999), Arp-T1 (actin related protein), and Arp-T2 (Heid et al., 2002) were observed in mammalian spermatozoa.

Therefore before the acrosome reaction occurs, actin severing proteins undergo rapid dephosphorylation. Phosphatases are activated by enhanced intracellular $Ca^{2+}$ concentration. Actin filaments constitute the final barrier to membrane fusion and acrosomal exocytosis (Brener et al., 2003).

## *Conclusion*

Remodelling of the actin cytoskeleton is related to a number of processes during sperm life such as capacitation, acrosome reaction, remodelling of plasma membrane domains, and fusion of gametes. Thus, clarification of its reassembly during these events is important for the better understanding of fertilization. However there is still a lot to be discovered in this field.

The study made by Carlsen et al. (1992) showed in human a significant decrease in mean sperm count from 113 x $10^6$/ml in 1940 to 66 x $10^6$/ml in 1990 and in seminal volume from 3,40 ml to 2,75 ml (Carlsen et al., 1992). Thus, it is expected that the importance of fertilization in vitro (IVF) will be rising in the future. Therefore, clarifying of the molecular mechanisms of fertilization processes could help to obtain better results in IVF and could be also used to help survive endangered species.

## *List of abbreviations*

AC – adenylyl cyclase

ADF – actin-depolymerizing factor

ADP – adenosin diphosphate

ATP – adenosin triphosphate

BSA – bovine serum albumin

cAMP – cyclic adenosin monophosphate

DAG – diacylglicerol

DNA – deoxyribonuclear acid

EGF – epidermal growth factor

F-actin – filamentous actin

G-actin – globular actin

$IP_3$ – inositol 1,4,5-triphosphate

IVF – *in vitro* fertilization

OAM – outer acrosomal membrane

PA – phosphatidic acid

PDE – cyclic nucleotic phosphodiesterase

PIP – phosphatidylinositol 4- phosphate

$PIP_2$ – phosphatidylinositol 4,5-bisphosphate

$PIP_3$ – phosphatidylinositol 1,4,5-triphosphate

$PI_3K$ – phosphatidylinositol 3-kinase

$PI_4K$ – phosphtidylinositol 4-kinase

PKA – protein kinase A

PKC – protein kinase C

PLC – phospholipase C

PLD – phospholipase D

PM – plasma membrane

R – receptor

SOC – store-operated calcium

STK – sperm tyrosine kinase

TK – tyrosine kinase

## References


**Aitken, R. J., Paterson, M., Fisher, H., Buckingham, D. W. and van Duin, M.** (1995). Redox regulation of tyrosine phosphorylation in human spermatozoa and its role in the control of human sperm function. *J Cell Sci* **108 ( Pt 5)**, 2017-25.

**Baldi, E., Luconi, M., Bonaccorsi, L., Krausz, C. and Forti, G.** (1996). Human sperm activation during capacitation and acrosome reaction: role of calcium, protein phosphorylation and lipid remodelling pathways. *Front Biosci* **1**, d189-205.

**Bedford, J. M. and Cross, N. L.** (1999). Sperm Capacitation. In *Encyclopedia of Reproduction*, vol. 4, pp. 597-602: Academic Press.

**Breitbart, H.** (2002). Intracellular calcium regulation in sperm capacitation and acrosomal reaction. *Mol Cell Endocrinol* **187**, 139-44.

**Breitbart, H.** (2003). Signaling pathways in sperm capacitation and acrosome reaction. *Cell Mol Biol (Noisy-le-grand)* **49**, 321-7.

**Brener, E., Rubinstein, S., Cohen, G., Shternall, K., Rivlin, J. and Breitbart, H.** (2003). Remodeling of the actin cytoskeleton during mammalian sperm capacitation and acrosome reaction. *Biol Reprod* **68**, 837-45.

**Cabello-Agueros, J. F., Hernandez-Gonzalez, E. O. and Mujica, A.** (2003). The role of F-actin cytoskeleton-associated gelsolin in the guinea pig capacitation and acrosome reaction. *Cell Motil Cytoskeleton* **56**, 94-108.

**Camatini, M., Casale, A. and Cifarelli, M.** (1988). Immunocytochemical identification of actin in rabbit spermiogenesis and spermatozoa. *Eur J Cell Biol* **45**, 274-81.

**Carlsen, E., Giwercman, A., Keiding, N. and Skakkebaek, N. E.** (1992). Evidence for decreasing quality of semen during past 50 years. *Bmj* **305**, 609-13.

**Castellani-Ceresa, L., Brivio, M. F. and Radaelli, G.** (1992). F-actin in acrosome-reacted boar spermatozoa. *Mol Reprod Dev* **33**, 99-107.

**Castellani-Ceresa, L., Mattioli, M., Radaelli, G., Barboni, B. and Brivio, M. F.** (1993). Actin polymerization in boar spermatozoa: fertilization is reduced with use of cytochalasin D. *Mol Reprod Dev* **36**, 203-11.

**Clarke, G. N., Clarke, F. M. and Wilson, S.** (1982). Actin in human spermatozoa. *Biol Reprod* **26**, 319-27.

**Cohen, G., Rubinstein, S., Gur, Y. and Breitbart, H.** (2004). Crosstalk between protein kinase A and C regulates phospholipase D and F-actin formation during sperm capacitation. *Dev Biol* **267**, 230-41.

**Davis, B. K., Byrne, R. and Bedigian, K.** (1980). Studies on the mechanism of capacitation: albumin-mediated changes in plasma membrane lipids during in vitro incubation of rat sperm cells. *Proc Natl Acad Sci U S A* **77**, 1546-50.

**Davis, B. K. and Gergely, A. F.** (1979). Studies on the mechanism of capacitation: changes in plasma membrane proteins of rat spermatozoa during incubation in vitro. *Biochem Biophys Res Commun* **88**, 613-8.

**de las Heras, M. A., Valcarcel, A., Perez, L. J. and Moses, D. F.** (1997). Actin localization in ram spermatozoa: effect of freezing/thawing, capacitation and calcium ionophore-induced acrosomal exocytosis. *Tissue Cell* **29**, 47-53.

**Eddy, E. M. and O'Brien, D. A.** (1994). The Spermatozoon. In *The Physiology of Reproduction*, (ed. E. Knobil and J. D. Neil), pp. 29-62. New York: Raven Press.

**Etkovitz, N., Rubinstein, S., Daniel, L. and Breitbart, H.** (2007). Role of PI3-kinase and PI4-kinase in actin polymerization during bovine sperm capacitation. *Biol Reprod* **77**, 263-73.

**Fouquet, J. P. and Kann, M. L.** (1992). Species-specific localization of actin in mammalian spermatozoa: fact or artifact? *Microsc Res Tech* **20**, 251-8.

**Galantino-Homer, H. L., Florman, H. M., Storey, B. T., Dobrinski, I. and Kopf, G. S.** (2004). Bovine sperm capacitation: assessment of phosphodiesterase activity and intracellular alkalinization on capacitation-associated protein tyrosine phosphorylation. *Mol Reprod Dev* **67**, 487-500.
**Galantino-Homer, H. L., Visconti, P. E. and Kopf, G. S.** (1997). Regulation of protein tyrosine phosphorylation during bovine sperm capacitation by a cyclic adenosine 3'5'-monophosphate-dependent pathway. *Biol Reprod* **56**, 707-19.
**Garbi, M., Rubinstein, S., Lax, Y. and Breitbart, H.** (2000). Activation of protein kinase calpha in the lysophosphatidic acid-induced bovine sperm acrosome reaction and phospholipase D1 regulation. *Biol Reprod* **63**, 1271-7.
**Garty, N. B. and Salomon, Y.** (1987). Stimulation of partially purified adenylate cyclase from bull sperm by bicarbonate. *FEBS Lett* **218**, 148-52.
**Heid, H., Figge, U., Winter, S., Kuhn, C., Zimbelmann, R. and Franke, W.** (2002). Novel actin-related proteins Arp-T1 and Arp-T2 as components of the cytoskeletal calyx of the mammalian sperm head. *Exp Cell Res* **279**, 177-87.
**Howes, E. A., Hurst, S. M. and Jones, R.** (2001). Actin and actin-binding proteins in bovine spermatozoa: potential role in membrane remodeling and intracellular signaling during epididymal maturation and the acrosome reaction. *J Androl* **22**, 62-72.
**Hyne, R. V. and Garbers, D. L.** (1979). Regulation of guinea pig sperm adenylate cyclase by calcium. *Biol Reprod* **21**, 1135-42.
**Chang, M. C.** (1984). The meaning of sperm capacitation. A historical perspective. *J Androl* **5**, 45-50.
**Lefievre, L., Jha, K. N., de Lamirande, E., Visconti, P. E. and Gagnon, C.** (2002). Activation of protein kinase A during human sperm capacitation and acrosome reaction. *J Androl* **23**, 709-16.
**Liu, D. Y., Martic, M., Clarke, G. N., Dunlop, M. E. and Baker, H. W.** (1999). An important role of actin polymerization in the human zona pellucida-induced acrosome reaction. *Mol Hum Reprod* **5**, 941-9.
**Liu, D. Y., Martic, M., Clarke, G. N., Grkovic, I., Garrett, C., Dunlop, M. E. and Baker, H. W.** (2002). An anti-actin monoclonal antibody inhibits the zona pellucida-induced acrosome reaction and hyperactivated motility of human sperm. *Mol Hum Reprod* **8**, 37-47.
**Millette, C. F.** (1999). Spermatozoa. In *Encyclopedia of Reproduction*, vol. 4, pp. 586-596: Academic Press.
**Moreno-Fierros, L., Hernandez, E. O., Salgado, Z. O. and Mujica, A.** (1992). F-actin in guinea pig spermatozoa: its role in calmodulin translocation during acrosome reaction. *Mol Reprod Dev* **33**, 172-81.
**Ochs, D. and Wolf, D. P.** (1985). Actin in ejaculated human sperm cells. *Biol Reprod* **33**, 1223-6.
**Pelletier, R., Trifaro, J. M., Carbajal, M. E., Okawara, Y. and Vitale, M. L.** (1999). Calcium-dependent actin filament-severing protein scinderin levels and localization in bovine testis, epididymis, and spermatozoa. *Biol Reprod* **60**, 1128-36.
**Peterson, R., Russell, L., Bundman, D. and Freund, M.** (1978). Presence of microfilaments and tubular structures in boar spermatozoa after chemically inducing the acrosome reaction. *Biol Reprod* **19**, 459-66.
**Peterson, R. N., Bozzola, J. J., Hunt, W. P. and Darabi, A.** (1990). Characterization of membrane-associated actin in boar spermatozoa. *J Exp Zool* **253**, 202-14.
**Rivlin, J., Mendel, J., Rubinstein, S., Etkovitz, N. and Breitbart, H.** (2004). Role of hydrogen peroxide in sperm capacitation and acrosome reaction. *Biol Reprod* **70**, 518-22.

**Scarlett, C. J., Lin, M. and Aitken, R. J.** (2001). Actin polymerisation during morphogenesis of the acrosome as spermatozoa undergo epididymal maturation in the tammar wallaby (Macropus eugenii). *J Anat* **198**, 93-101.
**Schmidt, A. and Hall, M. N.** (1998). Signaling to the actin cytoskeleton. *Annu Rev Cell Dev Biol* **14**, 305-38.
**Spungin, B., Margalit, I. and Breitbart, H.** (1995). Sperm exocytosis reconstructed in a cell-free system: evidence for the involvement of phospholipase C and actin filaments in membrane fusion. *J Cell Sci* **108 ( Pt 6)**, 2525-35.
**Talbot, P. and Kleve, M. G.** (1978). Hamster sperm cross react with antiactin. *J Exp Zool* **204**, 131-6.
**Virtanen, I., Badley, R. A., Paasivuo, R. and Lehto, V. P.** (1984). Distinct cytoskeletal domains revealed in sperm cells. *J Cell Biol* **99**, 1083-91.
**Visconti, P. E., Bailey, J. L., Moore, G. D., Pan, D., Olds-Clarke, P. and Kopf, G. S.** (1995a). Capacitation of mouse spermatozoa. I. Correlation between the capacitation state and protein tyrosine phosphorylation. *Development* **121**, 1129-37.
**Visconti, P. E., Moore, G. D., Bailey, J. L., Leclerc, P., Connors, S. A., Pan, D., Olds-Clarke, P. and Kopf, G. S.** (1995b). Capacitation of mouse spermatozoa. II. Protein tyrosine phosphorylation and capacitation are regulated by a cAMP-dependent pathway. *Development* **121**, 1139-50.
**Visconti, P. E., Westbrook, V. A., Chertihin, O., Demarco, I., Sleight, S. and Diekman, A. B.** (2002). Novel signaling pathways involved in sperm acquisition of fertilizing capacity. *J Reprod Immunol* **53**, 133-50.
**Vogl, A. W., Genereux, K. and Pfeiffer, D. C.** (1993). Filamentous actin detected in rat spermatozoa. *Tissue Cell* **25**, 33-48.
**von Bulow, M., Heid, H., Hess, H. and Franke, W. W.** (1995). Molecular nature of calicin, a major basic protein of the mammalian sperm head cytoskeleton. *Exp Cell Res* **219**, 407-13.
**von Bulow, M., Rackwitz, H. R., Zimbelmann, R. and Franke, W. W.** (1997). CP beta3, a novel isoform of an actin-binding protein, is a component of the cytoskeletal calyx of the mammalian sperm head. *Exp Cell Res* **233**, 216-24.
**Yagi, A. and Paranko, J.** (1995). Actin, alpha-actinin, and spectrin with specific associations with the postacrosomal and acrosomal domains of bovine spermatozoa. *Anat Rec* **241**, 77-87.
**Yanagimachi, R.** (1994). Mammalian Fertilization. In *The Physiology of Reproduction*, (ed. E. Knobil and J. D. Neil), pp. 189-280. New York: Raven Press.